AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph beginning on page 11, line 18, with the following amended paragraph:

Figure 5 is a graphical representation of K^b stabilisation on RMA-S by β_{C3}-amino acid substituted and parental peptides. Substitution at the N-terminal region of the peptide (a) disrupts K^b stabilisation, whereas substitution in the remaining middle and C-terminal end (b) results in equivalent or increased stabilisation. Serial dilutions of the indicated peptides were pulsed onto cold-induced RMA-S cells (26°C) which were then pulsed at 37°C. Stabilisation of K^b at the surface was detected using the monoclonal antibody Y-3 and analysed by flow cytometry. Data is shown as percentage of maximal SIINFEKL (SEQ ID NO: 1) response against peptide concentration (log scale).

Please replace the paragraph beginning on page 11, line 27, with the following amended paragraph:

Figure 6 is a graphical representation of K^b stabilisation on RMA-S by β_{C3}-amino acid substituted and parental peptide using the antibody 25-D1.16. Substitution of β-amino acids resulted in a decreased affinity for the K^b/SIINFEKL (SEQ ID NO: 1) specific antibody 25-D1.16. Data is shown as percentage of maximal wild type response against peptide concentration (log scale).

Please replace the paragraph beginning on page 12, line 2, with the following amended paragraph:

Figure 7 is a graphical representation of the titration of Parental SIINFEKL (SEQ ID NO: 1) against each CTL clone. As expected HSV2.3 control did not recognise the SIINFEKL (SEQ ID NO: 1) peptide. Data is shown as radioactive counts against peptide concentration.

Please replace the paragraph beginning on page 12, line 6, with the following amended paragraph:

Figure 8 is a graphical representation of the recognition of the parental SIINFEKL (SEQ ID NO: 1) and β_{C3}-amino acid analogues by CTL clones. Substitution at P2 resulted in a significant loss of recognition with all clones except for GA4.2. At P6 the β_{C3}-Glu containing the benzyl side-chain protecting group completely abolished CTL recognition. Peptides were pulsed onto I-3 cells, then co-cultured with CTLs. Supernatant was then removed and added to an IL-2 bioassay. Data is shown as percentage of SIINFEKL response (in the case of the control clone, HSV2.3, the data is shown as percentage of SSIEFARL (SEQ ID NO: 12) response).

Please replace the paragraph beginning on page 12, line 14, with the following amended paragraph:

Figure 9 is a graphical representation of the recognition of the parental SIINFEKL (SEQ ID NO: 1) and β_{C3}-amino acid analogues by CTL clone GA 4.2

Please replace the paragraph beginning on page 12, line 17, with the following amended paragraph:

Figure 10 is a graphical representation of an antagonist assay using the CTL clones (a) GA4.2 and (b) B3.1. β-analogues are co-administered with a suboptimal concentration of the parental SIINFEKL (SEQ ID NO: 1) peptide (dashed line). Supernatant of pulsed I-3 cells co-cultured with CTLs is then used in an IL-2 bioassay. Substitution at position P1 resulted in strong antagonism of B3.1 and weak antagonism of GA4.2. Substitution at P2 resulted a super agonist effect occurring in both clones. Both analogues mediated their response at extremely low concentrations indicating that only very low levels of analogue peptide are needed to alter signalling through the TCR.

Please replace the paragraph beginning on page 12, line 30, with the following amended paragraph:

Figure 12 is a graphical representation of parental SIINFEKL (SEQ ID NO: 1) degradation after 2 hrs as monitored by RP-HPLC at a wavelength of 214nm. The Chromatogram shows the fragmentation of the parental peptide. The MS results reveal the cleavage sites for the enzymatic degradation. Cleavage occurs at bonds marked *:

S*I*I*NF*E*KL (SEQ ID NO: 1)

Please replace the paragraph beginning on page 13, line 5, with the following amended paragraph:

Figure 13 is a graphical representation of the degradation of the peptide containing a β-Phe at P5 at time points 0, 2 and 12 hrs. At t=0 the full peptide can be seen. At t=2 hrs the peptide full peptide is reduced and other fragments can be seen. At t=12 hrs the full peptide has disappeared leaving only fragments. MS of the fragments reveals that cleavage did not occur after the P5 amino acid as it did for the parental peptide. * Indicates blocked cleavage site:

S*I*I*NF*E*KL (SEQ ID NO: 1)

Please replace the paragraph beginning on page 13, line 13, with the following amended paragraph:

Figure 14 is a graphical representation of the degradation of the peptide containing a β-Ile at P3 at time points 0 and 2 hrs. At t=0 the full peptide can be seen. At t=2 hrs most of the full peptide has disappeared, with three main fragments. MS of the fragments revealed that there was no cleavage at the P2-P3 bond, which was a site of cleavage for the parental peptide. * Indicates blocked cleavage site: S*I*I*NF*E*KL (SEQ ID NO: 1)

Please replace the paragraph beginning at page 13, line 19, with the following amended paragraph:

Figure 15 is a schematic representation of the presentation of natural (SEQ ID NOS: 11 and 12) and cryptic (SEQ ID NOS: 13 and 14) NY-ESO determinants following vaccination with NY-ESO 157-165 (SEQ ID NO: 11) and 157-167 (SEQ ID NO: 12) (taken from [1]).

Please replace the paragraph beginning at page 13, line 22, with the following amended paragraph:

Figure 16 is a schematic representation of: A) HLA A*0201/$ESO_{157-165}$ complex crystallizes in cubic form. B) 2.1Å electron density omit map of $ESO_{157-165}$ peptide. C) Cut-away view of $ESO_{157-165}$ (SEQ ID NO: 10) bound to the HLA-A2 antigen binding cleft, highlighting the exposed Met-4, Trp-5, Thr-7, Gln-8.

Please replace the paragraph beginning at page 14, line 1, with the following amended paragraph:

Figure 19 is a schematic representation of a SIINFEKL (SEQ ID NO: 1) peptide conformation.

Please replace the paragraph beginning at page 14, line 3, with the following amended paragraph:

Figure 20 is a schematic representation of the SIIN-βF-EKL (SEQ ID NO: 6) peptide conformation.

Please replace the paragraph beginning at page 14, line 5, with the following amended paragraph:

Figure 21 is a schematic representation of the SIINFEK-βL (SEQ ID NO: 8) peptide conformation.

Please replace the paragraph beginning at page 50, line 28, with the following amended paragraph:

The SIINFEKL (SEQ ID NO: 1)/K^b-restricted $CD8^+$ CTL clones B3.1, GA4.2 (Nikolic-Zugic, J., and Carbone F.R. (1990). The effect of mutations in the MHC Class I peptide binding groove on the cytotoxic T lymphocyte recognition of the Kb restricted ovalbumin determinant. Eur. J. Immunol. 20:2431), 149.13.13, 149.42.12 (Clark S. R., Barnden M., Kurts C., Carbone F.R., Miller J.F., Heath J.R. Characterization of the ovalbumin-specific TCR transgenic line OT-I: MHC elements for positive and negative selection. Immunol Cell Biol. 2000 Apr;78(2):110-7), and the herpes simplex viral specific clone HSV2.3 (Wallace, M. E., Keating, R., Heath, W R., Carbone, F. R. 1999. The cytotoxic T-cell response to herpes simplex virus type 1 infection of C57BL/6 mice is almost entirely directed against a single immunodominant determinant. J Virol. Sep;73(9):7619-26) were derived and maintained as described. All T cell hybrids were grown in commercial DMEM containing 10% fetal calf serum, L-glutamine and antibiotics (0.7mg/ml G418). CTL clones were used in recognition assays, and clones B3.1 and GA4.2 were used in antagonism assays. T hybrids were extensively phenotyped prior to use, including examination of T cell function and cell surface phenotype (flow cytometric staining for CD8; see Figure 2).

Please replace the paragraph beginning at page 54, line 16, with the following amended paragraph:

SIINFEKL (SEQ ID NO: 1)/H2-Kb Stabilisation assay

The procedure from the K^b stabilisation assay above was repeated with the monoclonal antibody 25-D1-16 that specifically binds to SIINFEKL (SEQ ID NO: 1)/H2-K^b complexes and not to other H2-K^b peptide combinations (Porgador, A., J. W. Yewdell, Y. Deng, J. R. Bennink, R. N. Germain. 1997. Localization, quantitation, and in situ detection of specific peptide-MHC class I complexes using a monoclonal antibody. *Immunity 6:715*).

Please replace the paragraph beginning at page 55, line 17, with the following amended paragraph:

I-3 adherent antigen presenting cells (APC) were pre-plated ~ 10^5 cells per well overnight at 37°C. APC were pulsed with 1nM of agonist SIINFEKL (SEQ ID NO: 1) peptide (this concentration gave 50% maximal stimulation of the T hybridomas see results figure 9) and incubated for 1 hr at 37°C. Graded concentrations (10^{-13}M to 10^{-6}M) of β-analogues were then added and incubated for an additional 1 hr. T-hybrid clones grown to ~ 10^6 cells/ml were washed and resuspended at a density of 10^5 cells per well, then transferred to wells containing the pulsed I-3 cells. T-hybrid clones and APC were then incubated in co-culture for 20-24 hrs. 50µl of co-culture supernatant was harvested from each well and added to the CTLL-2 bioassay as in the T-hybrid assay. Antagonism was detected as a decrease in the basal level of IL-2 production at a concentration of agonist peptide resulting in half maximal stimulation of the T hybrids.

Please replace the paragraph beginning on page 56, line 15, with the following amended paragraph:

The column was a Pharmacia µRPC octadecyl silica column of 3µM nominal particle size and 300Å pore size. The flow rate was 200µl/min and UV detection was used at 214nm, 254nm and 280nm. Values are given as area of peptide peak (214nm). Peptide amounts at T = 0 min were used as 100%. Peptide digests were fractionated and Electrospray ionisation

(ESI) Ion Trap Mass spectrometry was used to characterise the fragmentation pattern of SIINFEKL (SEQ ID NO: 1) and selected β-amino acid analogues.

Please replace the paragraph beginning at page 56, line 27, with the following amended paragraph:

The response to SIINFEKL (SEQ IDNO: 1) by CTLs in C57/BL6 mice is predominantly restricted through the MHC Class I allele H-2K^b (Porgador, A., J. W. Yewdell, Y. Deng, J. R. Bennink, R. N. Germain. 1997. Localization, quantitation, and in situ detection of specific peptide-MHC class I complexes using a monoclonal antibody. *Immunity 6:715*). To characterise the effect that β_{C3}-amino acids have on K^b stabilisation, mutant peptides of the SIINFEKL (SEQ ID NO: 1) peptide containing single β_{C3}-amino acid substitutions were synthesised (Table 4). The peptides were tested for binding to the surface K^b on the TAP mutant cell line RMA-S.

Please replace the paragraph beginning on page 57, line 4, with the following amended paragraph:

These cells express low levels of K^b (~ 5% - 10% of normal) at 37°C [19], but at incubation temperatures between 19° and 33°C expression of surface K^b is amplified (~ 50% of parental RMA cell line) (Ljunggren, H. G., N. J. Stam, J. J. C. Öhlén, P. Neefjes, M. T. Höglund, J. Heemels, T. N. Bastin, A. Schumacher, K. Kärre Townsend, H. L. Ploegh. 1990. Empty MHC class I molecules come out in the cold. *Nature 346:476)*. These cold-induced molecules are quickly removed from the surface when the cells are returned to 37°C unless stabilised by a K^b binding peptide. RMA-S cells were cultured overnight at 26°C and tested for K^b expression, cells were then pulsed with serial dilutions of each peptide for an additional 1 hr before being placed at 37°C for 2 hrs. Substitution at the N-terminus end of the peptide resulted in decreased binding affinity to the K^b molecule. However, substitution in the middle and C-terminus end resulted in equivalent or increased binding stabilisation of

cold stabilised Kb molecules relative to the SIINFEKL (SEQ ID NO: 1) peptide. Results were highly reproducible and representative data are shown in Figure 5.

Please replace the paragraph beginning at page 57, line 22, with the following amended paragraph:

The monoclonal antibody 25-D1.16 specifically binds to the SIINFEKL (SEQ ID NO: 1) peptide in conjunction with the K^b molecule. The SIINFEKL (SEQ ID NO: 1) peptide is mostly buried within the MHC cleft with only the side chains of residues at P4, P6 and P7 facing outwards from the surface of the complex. This peptide binding orientation was first predicted by an alanine substitution scan (Jameson S. C., Bevan M. J., Dissection of major histocompatibility complex (MHC) and T cell receptor contact residues in a Kb-restricted ovalbumin peptide and an assessment of the predictive power of MHC-binding motifs. Eur J Immunol. 1992 Oct;22(10):2663-7) of SIINFEKL (SEQ ID NO: 1) and then confirmed by the K^b/SIINFEKL (SEQ ID NO: 1) crystal structure (Fremont D. H., Stura E. A., Matsumura M., Peterson P. A., and Wilson I. A. Crystal structure of an H-2Kb-ovalbumin peptide complex reveals the interplay of primary and secondary anchor positions in the major histocompatibility complex binding groove. Proc Natl Acad Sci U S A. 1995 Mar 28;92(7):2479-83). The residues Asn, Glu and Lys of SIINFEKL (SEQ ID NO: 1) (P4, P6 and P7 respectively) are known to be involved in CTL recognition. To test the impact of incorporating β_{C3}-amino acids into SIINFEKL (SEQ ID NO: 1) on the orientation of these exposed residues, the RMA-S stabilisation assay was repeated with the antibody 25-D1.16. Changes in 25-D1.16 recognition have previously been correlated with altered TCR recognition (Porgador *et al*, 1997, *supra*), suggesting this antibody has TCR like binding specificity.

Please replace the paragraph beginning at page 58, line 10, with the following amended paragraph:

Substituting a β-amino acid into any part of the SIINFEKL (SEQ ID NO: 1) peptide resulted in a change in the orientation or composition of solvent exposed residues. The most vulnerable positions for substitutions were P2 and P5, which abolished recognition by the 25-D1.16 antibody. Substitutions at P4 and P8 had the least effect, while peptides with substitutions at P1 and P3 needed a 100-fold increase in concentration to reach the same level of recognition as SIINFEKL (SEQ ID NO: 1). The results from the analysis were highly reproducible and representative data are shown in Figure 6.

Please replace the paragraph beginning at page 58, line 22, with the following amended paragraph:

CTL clones (expressing different TCR Vβ/Vα segments) restricted by the H2-K^b molecule, and specific to the SIINFEKL (SEQ ID NO: 1) peptide were tested for their ability to recognise the various β_{C3}-amino acid substituted analogues (Table **X**). A titration of the parental SIINFEKL (SEQ ID NO: 1) peptide on each clone was completed to determine the optimal peptide concentration for CTL recognition (Figure 7). I-3 cells were cultured overnight then pulsed with 1 μM of each of the β_{C3}-amino acid analogues for 1 hr. CTL clones were placed in co-culture with pulsed I-3 cells for 20-24 hrs. Supernatant from co-culture containing secreted IL-2 is then added to the IL-2dependent CTLL-2 cells. CTTL-2 proliferation is then measured by 3H-Thymydine incorporation and counted on a scintillation counter.

Please replace the paragraph beginning at page 59, line 1, with the following amended paragraph:

Differences were observed in the effect of substitutions at different positions. Analogues

with substitutions at P1, P3, P4, P5 and P8 were least affected with considerable recognition from the CTL GA4.2, with approximately 50% recognition by B3.1 and 30% by 149.13.13. Although recognition by 149.42.12 was almost non-existent when compared to the parental peptide. The analogue with a P2 substitution resulted is significantly less recognition by all clones except GA4.2. At P6 (residue with side chain protecting group), no response was observed. The response seen in the control CTL clone HSV2.3 (Herpes Simplex Virus specific clone) is mainly mediated by the SSIEFARL (SEQ ID NO: 12) peptide, as expected this peptide was not recognised by the K^b/SIINFEKL (SEQ ID NO: 1) restricted clones. Concanavalin A was used to induce maximal IL-2 secretion in CTL clones as an indicator of CTL functionality.

Please replace the paragraph beginning at page 59, lines 13-21, with the following amended paragraph:

The effect of substituting β_{C3}-amino acids on CTL recognition had some correlation with stability of peptides to K^b. For instance β_{C3}-Ile at P2, which had the lowest affinity for K^b and also the least recognition by all CTL clones. Additionally, β_{C3}-Phe at P5, which had the strongest stabilising effect on K^b also had the most wide spread response to CTL clones. These results indicate that substitutions of β_{C3}-amino acids at individual positions of the SIINFEKL (SEQ ID NO: 1) peptide generally decrease CTL recognition. This effect is dependent on the position of the substitution and does show a subtle correlation with stability of the K^b/peptide complex. Results were reproducible and representative data are shown in Figures 8 and 9.

Please replace the paragraph beginning at page 59, line 28, with the following amended paragraph:

The antagonist assay was used to determine if the incorporation of β_{C3}-amino acids could alter the normal signalling and IL-2 secretion even in the presence of the agonist peptide.

The peptide analogues were tested in an assay designed to distinguish TCR antagonism from competition with MHC binding. This involves prepulsing the target cells with a suboptimal dose of the SIINFEKL (SEQ ID NO: 1) peptide before incubating them with the β-analogues and CTLs. The suboptimal concentration chosen of the natural SIINFEKL (SEQ ID NO: 1) peptide was 1 nM which was based on the SIINFEKL (SEQ ID NO: 1) titration assay (Figure 7) where this concentration induced 50% of the maximal IL-2 production in these co-cultures. I-3 cells were pulsed with 1 nM of the parental SIINFEKL (SEQ ID NO:1) peptide. β-amino acid substitutions were then titrated in. CTL clones GA4.2 and B3.1 were then placed in co-culture with pulsed I-3 cells, and after incubation supernatant from this co-culture was removed and placed into an IL-2 bioassay as in the CTL recognition assay.

Please replace the paragraph beginning at page 60, line 10, with the following amended paragraph:

The incorporation of β_{C3}-amino acids into the SIINFEKL (SEQ ID NO: 1) peptide resulted in two analogues that could modify the parental SIINFEKL (SEQ ID NO: 1) response. Substituting β-Ile at P2 produced an analogue that increased the relative IL-2 secretion of both clones. In the previous CTL recognition assay the substitution of β-Ile at P2 resulted in decreased IL-2 secretion in both B3.1 and GA4.2 cells (Figures 8[,] and 9), however when this peptide is co-administered with the parental SIINFEKL (SEQ ID NO: 1) peptide it increased the IL-2 secretion above the SIINFEKL (SEQ ID NO: 1) basal response (Figure 10). Conversely, the second analogue, β-Ser at P1, was a strong antagonist of B3.1 and a weaker antagonist of GA4.2. In the previous CTL recognition assay β-Ser peptide analogues stimulated CTL clones with a relatively high efficacy (Figure 8), but when co-administered with the parental SIINFEKL (SEQ ID NO: 1) peptide it resulted in extensive antagonism of the parental SIINFEKL (SEQ ID NO: 1) response. The fact that both these analogues altered the response in both cell lines demonstrates that the modifying TCR signalling by using β-analogues may not be a monoclonal event. This antagonism of the parental response was observed to occur down to 0.1 pM in both CTL clones. Results were reproducible and representative data are shown in Figure 10.

Please replace the paragraph beginning at page 60, line 31, with the following amended paragraph:

Since β-amino acids are resistant to proteolysis, the influence of peptide modification on stability against proteolysis was tested by monitoring the degradation of the peptides in mouse blood serum. Degradation kinetics were followed by reverse phase-HPLC (RP-HPLC) analysis using corresponding peak area for peptide quantification. The parental SIINFEKL (SEQ ID NO: 1) peptide was degraded rapidly, with only 5% of the peptide being recovered after 2 hrs. For the β_{C3}-analogues, the amount of degradation was highly dependent on the position of the substitution. Limited stabilisation occurred for peptides with substitutions at P3 and P4 with 8% and 7% recovery respectively (Figure 11). For the substitution at P1, 10% of the initial peak was recovered. However, a significant resistance to proteolytic degradation was formed with substitutions at P2, P5, and P8. For β-Phe at P5 and β-Leu at P8 over 25% of the initial peak was recovered after 2 hrs.

Please replace the paragraph beginning at page 61, line 12, with the following amended paragraph:

From the HPLC analysis it was observed that some fragments of degraded β-Ile (P3) and β-Phe (P5) peptides were accumulating over time (Figures 12 -14). Mass Spectrometry and MS/MS were carried out on these peptide fragments as well as the parental SIINFEKL (SEQ ID NO: 1) fragments. Together the results revealed the cleavage points from serum proteolysis, and also showed that the incorporation of the β-amino acid stopped cleavage at the position of substitution. Representative results are shown in Figures 12, 13 and 14.

Please replace the paragraph beginning at page 65, line 9, with the following amended paragraph:

The structure of HLA-A2 complexed to $ESO_{157-165}$ (SLLMWITQC) (SEQ ID NO: 10) to 2.1Å resolution has been solved (Fig. 16). The electron density for the peptide ligand is unambiguous and clearly shows the solvent accessibility of Met-4, Trp-5, Thr-7 and Gln-8, indicating that these residues would play a key role in TcR recognition. The Cys-8 residue is buried and participates in anchoring interactions with the hydrophobic F pocket. This indicates that the thiol is not critical for binding, however, comparison of this structure to A9, V9 or I/L9 analogs should reveals the role the thiol plays in modulating the antigen binding cleft conformation.

Please replace the paragraph beginning at page 67, line 23, with the following amended paragraph:

A similar β-amino acid scan, to that shown for the SIINFEKL (SEQ ID NO: 1) epitope is performed for $ESO_{157-165}$ and $ESO_{157-167}$. The effects of β-amino acid substitution on proteolytic stability, generation of immunogenic determinants and anti-tumor cytotoxic T lymphocyte-cross-reactivity are examined. Presentation of $ESO_{157-165}$ without liberation of cryptic epitopes associated with N-terminal trimming is obtained. Other epitopes may be studied as they come on line. Modification of the oxidatively sensitive Met and Cys residues is examined. C-terminally modified analogues are designed by replacing the Cys residue with the isosteric L-Amino butyric acid (Abu) or Ser. The structure allows the rational replacement of this residue to ensure the fidelity of the MHC-peptide complex and the elements important for TcR recognition of tumor cells are maintained.

Please replace the paragraph beginning at page 72, line 12, with the following amended paragraph:

Effector cells were obtained from C57BL/6 mice immunised with 100μg SIINFEKL (SEQ ID NO: 1), SIINβFEKL (SEQ ID NO: 6) or SIINFEKβL (SEQ ID NO: 8) in complete Freunds adjuvant 7 days previously. Spleen cells were depleted of erythrocytes by treatment with tris-buffered ammonium chloride and antigen specific T cells were restimulated in vitro for 13 days using irradiated SIINFEKL (SEQ ID NO: 1) pulsed splenocytes from naïve C57BL/6 mice.

Please replace the paragraph beginning at page 72, line 18, with the following amended paragraph:

Target cells used in CTL assays were EL4 (H-2b) thymoma cells or EG-7 (EL4 transfected with the Ovalbumin antigen facilitating physiological presentation of the native SIINFEKL (SEQ ID NO: 1) determinant). Cells were labelled by resuspending in 200μL 5% FCS v/v in RPMI containing 200μCi 51 Cr (Amersham) and incubated for 2 hours at 37°C. After washing cells 3 times, concentration was adjusted to 10^5 cells/mL with RPMI and cells dispensed in 100μL aliquots into 96-well U-bottom TC plates. Serial dilutions of 100μL aliquots of effector cells were added in triplicate. After four hours incubation at 37°C/5%CO2, 25μL of the supernatant of each well was harvested onto Luma plates (Packard) and assayed for ^{51}Cr by a γ-counter (Packard Topcount NXT). The specific ^{51}Cr release at each effector: target ratio was calculated by subtraction of the cpm released spontaneously in wells containing target cells incubated with medium only. These values were then expressed as a percentage of cpm representing ^{51}Cr released in samples from wells in which the target cells were incubated in 1% Triton X-100 (Total releasable cpm). Spontaneous release ranged from 1 to 10% of total releasable cpm. Data are presented as the mean of the values obtained from triplicate cultures.

Please replace the paragraph beginning at page 73, line 6, with the following amended paragraph:

Recombinant H-2K^b molecules were expressed in *E. coli* as inclusion bodies as described [Garboczi, D.N., Madden, D.R. & Wiley, D.C. Five viral peptide-HLA-A2 co-crystals. Simultaneous space group determination and X-ray data collection. *J Mol Biol* **239**, 581-7 (1994)] using the BL21 (RIL) strain of *Escherichia coli*. The class I heavy chain was modified by the removal of the leader sequence, transmembrane region and cytosolic tail (amino acids 1-276 of the mature protein sequence). cDNA encoding this region was ligated into the bacterial expression vector pET, and transformed into the BL21 (RIL) strain of *Escherichia coli*. At an A600 of 0.6, cultures were induced with 1mM of isopropyl-1-thio-β-D-galactopyranoside (IPTG) for 12 hours, bacteria were lysed in 50mM Tris-HCl pH 8.0, 1% TritonX-100, 1% Sodium deoxycholate, 100mM NaCl and 10mM DTT. Inclusion bodies were isolated by centrifugation after washing with 50mM Tris-HCl, 0.5% TritonX-100, 100mM NaCl, 1mM NaEDTA, 1mM DTT, pH 8.0, and washing in 50mM Tris-HCl, 1mM NaEDTA, 1mM DTT, pH 8.0, and then solubilized in 25mM MES, 8M Urea, 10mM NaEDTA, pH 6.0 with the protease inhibitors 1μg/ml Pepstatin A and 200μM phenylmethylsulfonyl fluoride (PMSF). Recombinant protein (60mg Kb heavy chain and 20mg β2m) was refolded with 30mg of the peptides (SIINβFEKL (SEQ ID NO: 6) and SIINFEKβL (SEQ ID NO: 8)) in the presence of 3M guanidine-HCl, 10mM NaAcetate, and 10mM NaEDTA, pH 4.2, over 24 hours in 0.1M Tris, 2mM EDTA, 400mM L-Arginine-HCl, 0.5mM Oxidized Glutathione, 5mM Reduced Glutathione pH 8.0 at 4°C. Following refolding, protein was dialyzed overnight against Milli Q using a 6-8,000 kDa MWCO dialysis membrane (Spectrum, California, USA). Protein was concentrated by ion exchange on a DE52 column (Whatman, Maidstone, Kent, U.K.), and subsequently purified by size exclusion on a Superdex 75pg gel filtration column (Amersham Pharmacia Biotech, Uppsala, Sweden), and a final ion exchange on a MonoQ HR 5/5 column (Amersham Pharmacia Biotech). Quantitative analysis was based on comparisons to BSA protein standards using SDS-polyacrylamide gel electrophoresis. Protein was concentrated to 3mg/ml for use in crystallization trials.

IN THE SEQUENCE LISTING:

Please replace the Sequence Listing currently of record(pages 1-5) with the Substitute Sequence Listing (pages 1-5) submitted herewith.